non-infectious SV40 DNA in its genome nor at least one additional oncogene. Additionally, the Examiner states that Ohnuki does not teach at least one defect in the origin of replication or in the *in vitro* process by which the tumor cell incorporates the DNA encoding at least one immortalizing oncocgene into a non-immortalized epithelial tumor cell. The Examiner additionally states that Ohnuki lacks the method step of incorporating DNA via microinjection, which is performed after the step of carrying out a primary expansion of the epithelial tumor cells comprising the step of culturing in a medium with epidermal growth factor on the extracellular matrix, collagen coated tissue flasks.

To correct the defects of Ohnuki, the Examiner alleges that Garcia teaches an autologous, disseminated immortalized rabbit mammary epithelial tumor cells which has integrated in its genome or another replicative genetic element the DNA encoding the early region (the large T antigen) of non-infectious SV40 DNA and which contains at least one defect in the origin of replication. The Examiner further alleges that Garcia continues to teach an epithelial tumor cell that has integrated in its genome at least one additional oncogene, wherein the additional oncogene is c-Ha-ras. The Examiner maintains that Garcia teaches the *in vitro* process by which the tumor cell incorporated the DNA encoding at least one immortalizing oncogene. The Examiner alleges that the incorporation step comprises microinjection, which was performed after the step of carrying out a primary expansion of the epithelial tumors cells. The Examiner states that the primary expansion comprises the step of culturing in a medium that comprises epidermal growth factor on the extracellular matrix, collagen coated tissue flasks.

The Examiner then concludes that based upon the teachings of Ohnuki and Garcia that it would have been obvious to use the cell lines of Ohnuki to establish a metastatic cell line suitable for studying the immortalizing and transforming potential of known and candidate genes for epithelial cells motivated by Ohnuki, Garcia and Change that the establishment of such a cell line could be readily made and successfully propagated in order to conduct experiments for the long term study of metastasis in many assay systems. The Examiner alleges that Chang discloses that human epithelial cells can be used to study epithelial cell biology, especially differentiation, which allegedly provides the motivation to combine Ohnuki and Garcia.

Applicants respectfully traverse this rejection because the Examiner has used applicants' own disclosure and impermissible hindsight to combine Ohnuki, Garcia and Chang to arrive at the claimed invention. Additionally, the Examiner has used the same rationale as she used for the rejections based on Carney as the primary reference but now relies on Ohnuki and Chang to support her rejections.

The present invention provides immortalized non-SCLC epithelial tumor cells that are derived from the earliest metastasizing cells which have conserved the phenotype of the residual tumor cells present in the patient. See the paragraph bridging pages 4 and 5 of the specification. It is important to recognize these cells at this very early stage and generate quantities of them to analyze the early stages of cancer for identification and therapeutic methods. These epithelial tumor cells with metastatic potential have a clearly different expression pattern of surface markers as compared to primary tumor cells. The Examiner is referred to page 2, last four lines of the specification. Accordingly, the cells of the present invention are clearly distinct from primary tumor cells described in Ohnuki, which are human prostatic adenocarcinoma cells. Therefore, the skilled artisan would not have combined the Ohnuki disclosure with Garcia and Chang to arrive at the claimed immortalized non-SCLC epithelial tumor cells which express an immortalizing oncogene.

Additionally, Ohnuki clearly teaches away from the claimed invention because the metastatic cell lines disclosed by Ohnuki are obtained from a patient suffering from undifferentiated Grade IV adenocarcinoma of the prostate metastatic to bone, as shown on page 524 of Ohnuki, right hand column, first paragraph of the "Materials and Methods" section. These cells are the described PC-3 cells. A further cell line, namely PC-5-Pl, was obtained by perineal biopsy from a primary prostatic adenocarcinoma (Grade IV)). Accordingly, both described cell lines (PC-3 and PC-5-Pl) are derived from an adenocarcinoma. Furthermore, it is of particular interest that on page 527, left column, first paragraph, lines 1-5 of the "Discussion," Ohnuki discloses that the chromosome profile of the PC-3 is characteristic of a poorly differentiated advanced human neoplasm. In particular, PC-3 is aneuploid and is characterized by the absence of chromosomes 2, 3, 5 and 15. Consequently, these cells cannot be characterized as being derived from the earliest metastasizing cells, as it is the case for the cells of the present invention.

Accordingly, the cells described in Ohnuki are distinct from the cells of the present invention, and a person skilled in the art at the priority date of the present invention would

not have been motivated to combine the teachings of Ohnuki with Garcia and Chang to arrive at the presently claimed cells.

The Examiner has cited Chang in combination with Ohnuki and Garcia but all that Chang provides are basic protocols for SV40 infections. Again, this reference does not provide for an immortalized, non-small cell lung cancer epithelial tumor cell or a method to generate such a cell. Chang does not disclose the transformation of cells as claimed in the present invention. It is of particular interest in this context that Chang provides for an overview of *in vitro* transformation of human epithelial cells. Nowhere does Chang disclose the transformation of non-small lung cancer eptithelial cells. Nor does Chang provide for the SV40 transformation of human bone marrow-derived cells.

Furthermore, Chang even teaches away from the claimed invention as shown on page 186, left column, second paragraph, lines 1-6, where it is recites that

> "[a]lthough it is generally accepted that the SV40 large T-antigen (and maybe the small t-antigen) is important for both the immortalization and malignant transformation of mouse cells, it is not known whether it is equally important for both these functions in SV40-transformed human cells..." (emphasis added)

Chang's disclosure further supports applicants' position by stating that the T-antigen is not involved in immortalization of SV40-transformed cells derived from simple epithelia. See Chang, page 186, left column, second complete paragraph, lines 1-10, that recites

> "[w]hilest T-antigen may play a direct role in the immortalization of SV40 transformed human keratinocytes (and other human cell types also committed to terminal differentiation), where crisis is non-existent or transient, it is less likely to be directly involved in the immortalization of SV40-transformed cells derived from simple epithelia (e.g. breast, colon) where crisis, after an extended lifespan in vitro, is the rule and escape from this degenerative state is a very rare event."

Applicants contend that this passage further teaches away from the presently claimed cells and does not provide the motivation to combine Ohnuki and Garcia to prepare the claimed invention. In this regard, Chang suggests that the large T- and small t-antigen are not important, and not even required for the immortalization of bone marrow derived human cells but the present invention shows that that is not case. However, the present inventors surprisingly have found that the T-antigen, as an immortalizing oncogene, is

essential for the immortalization of micrometastatic cells when transforming the cells with SV40.

In addition to the above arguments, applicants believe that the obviousness rejection based on Ohnuki in view of Garcia and Chang is improper. As argued in previous responses, Garcia does not disclose the transformation of a tumor cell but rather Garcia describes the transformation of a normal epithelial cell, and therefore the immortalization of a non-tumor cell. Furthermore, Garcia discloses the transformation of a rabbit cell by micro-injecting SV40 viral DNA and/or the human oncogene Ha-ras. In this regard, Garcia stresses on page 1980, left-hand column, first paragraph of discussion, second and third paragraphs:

> "When injected alone, these molecules were unable to transform rabbit mammary cells. The combination of SV40 DNA and activated c-Ha-ras gene, however, induced drastic changes in the micro-injected cells" (emphasis added)

In addition, on page 1974, right-hand column, last sentence of the introduction, Garcia points out:

> "An immortalized cell line obtained after injecting SV40 DNA into primary cells retained some but not all of the differentiation markers of mammary secretory cells from pregnant rabbits, whereas a cell line fully transformed by SV40 and the activated human c-Ha-ras DNA became tumorigenic." (emphasis added)

Therefore, Garcia teaches that tumorigenic cells can be obtained form normal epithelial cells by co-injecting SV40 and the human oncogene c-Ha-ras.

Further, there is simply no motivation to utilize the method of Garcia to make metastatic cell lines of Ohnuki's cells.

Applicants respectfully disagree with the Examiner's rationale for combining the cited prior art and for utilizing impermissible hindsight to construct the present rejection based upon Applicants' own disclosure. When combining elements to make out a *prima facie* case of obviousness, the Examiner is obliged to show by reference to specific evidence in the cited references that there was (i) a suggestion to make the combination and (ii) a reasonable expectation that the combination would succeed. Both the suggestion and reasonable expectation must be found within the prior art, and not be gleaned from Applicants' disclosure. *In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991); *In re Dow*

Chemical Co., 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). The Examiner has failed to support the alleged case of *prima facie* obviousness.

Obviousness "'cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination.'" *In re Fine*, 5 USPQ2d 1596, 1599 (Fed. Cir. 1988), *citing ACS Hosp. Sys. v. Montefiore Hosp.*, 221 USPQ 929, 933 (Fed. Cir. 1984). It is applicants' position that the combination of the prior art fails to provide a suggestion to make the present invention.

The Examiner reminds applicants that their previous arguments were unpersuasive because the claims are directed to a product and not a method of making the claimed product. But applicants respectfully remind the Examiner that her rejections are based on combining the characteristics of the cells of Ohnuki, the methods to immortalize and incorporate DNA and culture cells of Garcia motivated by the methods taught by Chang. But as the arguments provided above, Chang fails to provide the motivation to combine these prior art, and even teaches against doing so. For all of the reasons and all of the arguments presented above, this rejection should be withdrawn.

2. Claims 1-12, 16-22, 31 and 38

Claims 1-12, 16-22, 31 and 38 are alleged to be obvious over Ohnuki *et al.* ("Ohnuki") in view of Garcia *et al.* ("Garcia"), Blankenstein *et al.* ("Blankenstein") and Chang *et al.* ("Chang"). The Examiner applies Ohnuki, Garcia and Chang as above and Blankenstein to teach the transfer of single cytokine genes into cancer cells. The addition of Blankenstein fails to cure the deficiencies in the primary references, and in view of the above arguments directed to the combination of the primary references, it is requested that this rejection be withdrawn.

3. Claims 1-10, 16-22, 31 and 38

Claims 1-10, 16-22, 31 and 38 are alleged to be obvious over Ohnuki *et al.* ("Ohnuki") in view of Garcia *et al.* ("Garcia"), Chang *et al.* ("Chang") and the Sigma Cell Cutlure Catalogue and Price List ("Sigma"). The Examiner applies Ohnuki, Garcia and Chang as above, and Sigma to teach to availability of growth factor supplements for use in the culture medium. The addition of Sigma fails to cure the deficiencies in the

primary references, and in view of the all of the above arguments directed to the combination of the primary references, it is requested that this rejection be withdrawn.

4. Claims 1-10, 16-22, 33, 34 and 38

Claims 1-10, 16-22, 33, 34 and 38 are alleged to be obvious over Ohnuki *et al.* ("Ohnuki") in view of Garcia *et al.* ("Garcia"), Chang *et al.* ("Chang") and Gottlinger *et al.* ("Gottlinger"). The Examiner applies Ohnuki, Garcia and Chang, as above and Gottlinger to teach to epithelial surface antigens and adjuvants suitable for mounting an immunological response. The addition of Gottliner fails to cure the deficiencies in the primary references, and in view of the all of the above arguments, it is requested that this rejection be withdrawn.

CONCLUSION

Applicants kindly request consideration of the arguments presented herein. Applicants submit that this application is in condition for allowance, and they solicit an early indication to that effect. Should the Examiner believe that further discussion of any remaining issues would advance the prosecution, a telephone call to the undersigned, at the telephone number listed below, is courteously invited.

Respectfully submitted,

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Date

Jayme A. Huleatt
Reg. No. 34,485

FOLEY & LARDNER
3000 K Street, N.W.
Suite 500
Washington, D. C. 20007-5109
(202) 672-5300